GE Healthcare

Ettan microLC

Making Your First Runs

User Manual

Ettan

Important user information

All users must read this entire manual to fully understand the safe use of Ettan™ microLC

WARNING!

The WARNING! sign highlights instructions that must be followed to avoid personal injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION!

The Caution! sign highlights instructions that must be followed to avoid damage to the product or other equipment. It is important not to proceed until all stated conditions are met and clearly understood.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certifying

This product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.
The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

WARNING!

This is a Class A product. In a domestic environment this product may cause radio interference in which case the user may be required to make adequate measures.

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1 About this guide

This guide is written for users who are not familiar with UNICORN™ software and Ettan™ microLC. Here you will learn the basics of UNICORN and how to operate Ettan microLC from UNICORN.

UNICORN is a software package for control and supervision of the Ettan microLC chromatography system. It runs on a PC under Windows™, and includes hardware for interfacing the controlling PC to the chromatography liquid handling parts of Ettan microLC.

In this guide you will learn how to:

- Create methods.
- Prepare the system for runs.
- Perform runs.
- Make simple evaluations.
- Make reports.

Follow the guide from page-to-page in front of the computer. Your time will be well spent.

Note: To follow the instructions it is not necessary to read the comments (written with smaller font) containing additional information.

1.1 Pre-requisites

The system and the software must be installed and functioning and the monitor and pump calibrated as described in the separate Installation guide.

IMPORTANT! Before using Ettan microLC, read all the safety information in section *1.2* in *Ettan microLC System Manual.*

1.2 Typographical conventions

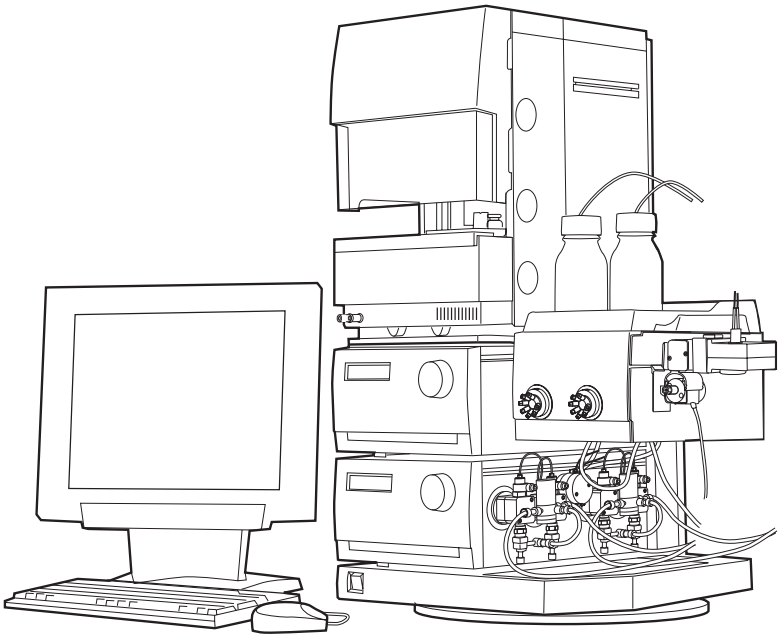
Software menu commands, dialog box prompts, hardware controls and indicators are identified in the text by bold text. A colon separates menu levels, thus **File:Open** refers to the **Open** command in the **File** menu.

2 The system and the software

2.1 General

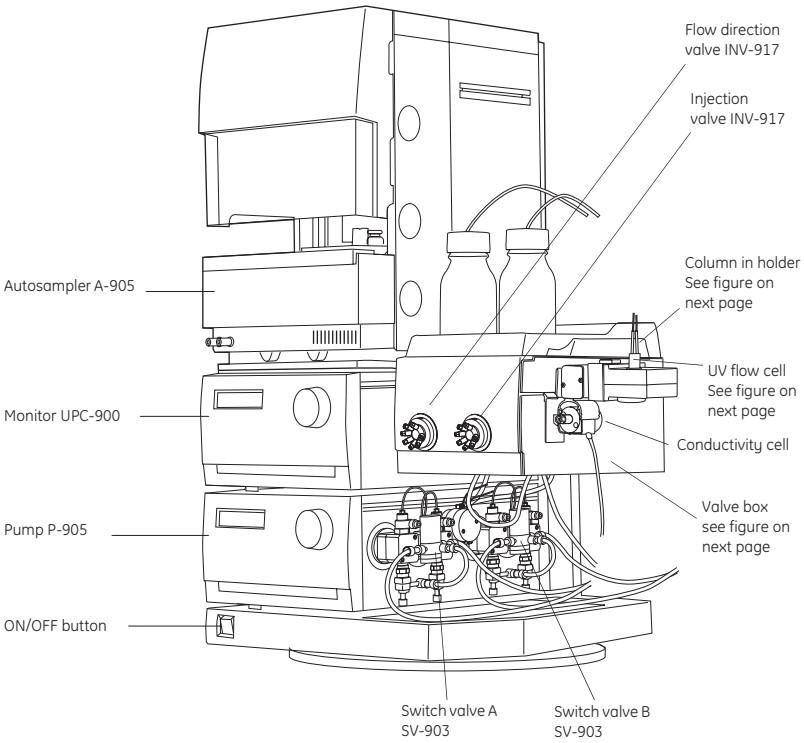
Ettan microLC is a fully automated liquid chromatography system designed for analytical applications. The separation unit of the chromatography system consists of three main modules. They are:

- Pump P-905, including a flow splitter for gradient formation at low flow rates.
- Monitor UPC-900, a combined UV and conductivity monitor.
- Autosampler A-905, for microtiter plates.



Components, such as the mixer, the flow splitter and the injection valve, are mounted in the valve box to the right.

The separation unit is controlled from the UNICORN software.

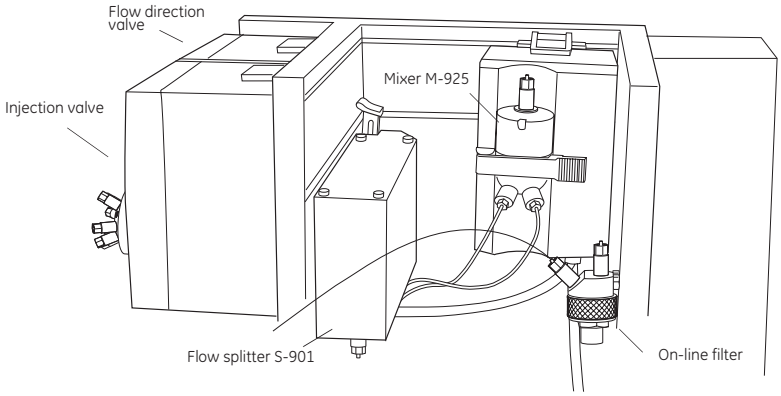

Flow direction valve INV-917
Injection valve INV-917
Column in holder See figure on next page
Autosampler A-905
UV flow cell See figure on next page
Monitor UPC-900
Conductivity cell
Valve box see figure on next page
Pump P-905
ON/OFF button
Switch valve A SV-903
Switch valve B SV-903

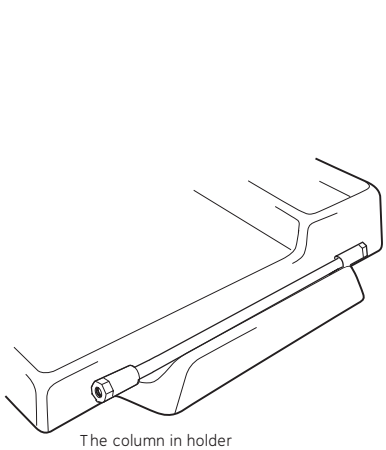
Pump P-905 and Monitor UPC-900 can also be controlled individually from the modules, without UNICORN software. In this guide, however, you will only learn how to operate the chromatography system from UNICORN.

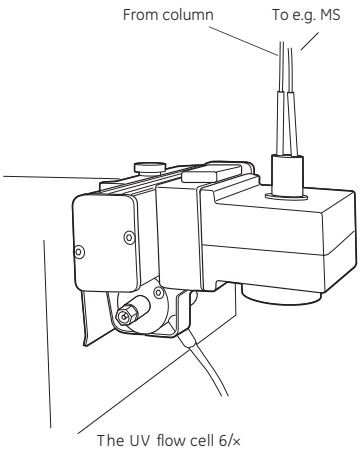
Switch on the chromatography system with the ON/OFF button located on the front of the base platform to the bottom left.

2.1.1 The valve box

The valve box contains the flow direction and the injection valve, the flow splitter, the mixer and the on-line filter.



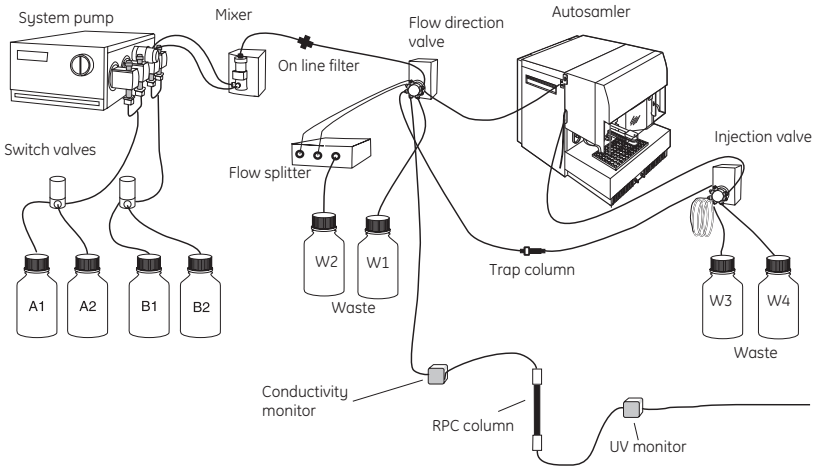

The column in holder



The UV flow cell 6/×

Comment:

The flowpath between the different modules and components in the separation unit is shown and described below. It is not necessary to go through this in detail to make your first runs.



1 The pump has 4 pump heads, two for pump A and two for pump B. Pump A is the one closest to the front.

2 The buffer solutions are pumped through switch valves, and further to a mixer. Inlet A1 and B1 are placed in buffer A and B respectively.

3 The flowpath continues from the pump to the mixer and forward via an on-line filter to the flow direction valve.

4 The flowpath passes through the flow splitter which is connected between ports 1 and 2 on the flow direction valve.

5 A trap column is connected between the flow direction valve and the injection valve.

6 A sample loop is connected between ports 2 and 6 on the injection valve. The sample loop is filled manually using a syringe. For manual filling connect a fill port to port 3 on the Injection valve.

7 After the injection valve, the flow is directed to the separation column via an autosampler and the flow direction valve, and then forward to the conductivity cell and the UV cell.

8 The flowpath finally continues to waste or to an MS instrument (optional).

2.2 UNICORN overview

1 Switch on the computer. Log in to Windows by first pressing **Ctrl-Alt-Del,** and then clicking on **OK.** After a while the Windows desktop appears.

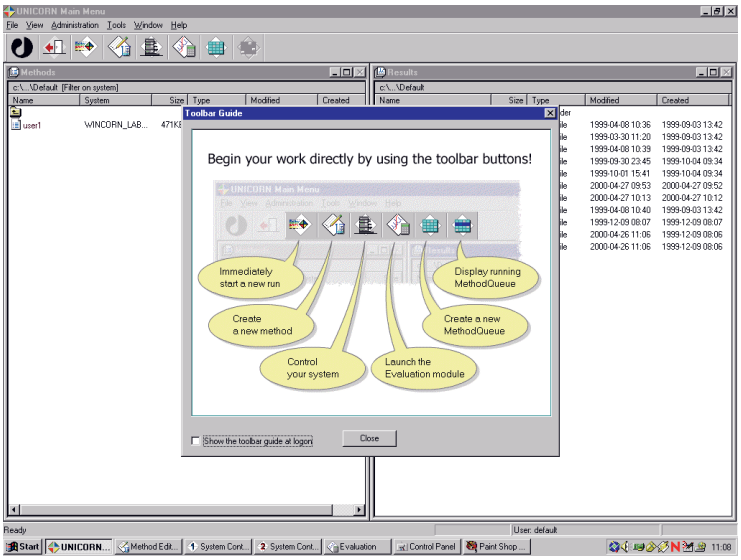
2 Start UNICORN by double-clicking on the UNICORN icon.

3 An information window appears during start-up.

4 When the **Logon** dialog appears, select a user from the **Users** list and enter the password. If you log in for the very first time, select user **default** and enter the password **default**. Click **OK**.

Note: You should enter users and individual passwords before starting using Ettan microLC on a regular basis.

5 Eventually, the UNICORN **Main Menu** window appears on the screen. At delivery, a Toolbar guide is displayed providing a quick guide on how to use the toolbar items. The Toolbar guide isinhibited by unchecking the square at the bottom left. Click **Close.**



6 The **Main Menu** window is the central part of UNICORN. From this window you can navigate through the whole control system. The **Main Menu** window is mainly used for file handling. In the **Methods** window to the left in Main menu, all method files that you create are displayed. A method file contains a series of instructions for controlling a run. In the **Results** window to the right, all result files are displayed. A result file is the result from a run, including all documentation (e.g. the method used) and the generated chromatogram.

In general, UNICORN consists of 4 different modules of which the Main menu is one. The other modules are represented by icons in the Toolbar. These modules are:

- New method — opens a dialog window for creating new methods.

- System control — opens a dialog window for controlling the system and running your methods.

- Evaluation — opens a dialog window for evaluating your results.

A module is opened by clicking on its icon.

Additional buttons are provided in the Toolbar. These are:

- Instant run — opens a dialog window where you directly can choose a template method to run. This is handy for starting routine runs instantly.

- Logon/Logoff — opens a dialog window to control the logon/logoff process.

- about UNICORN — opens an information window about the UNICORN version and how to contact GE Healthcare via the world wide web (internet).

- Method Queue — opens a dialog window for defining a new Method Queue.

- Existing Method Queue — opens a dialog window for showing the Method Queue that is running.

2.3 Help

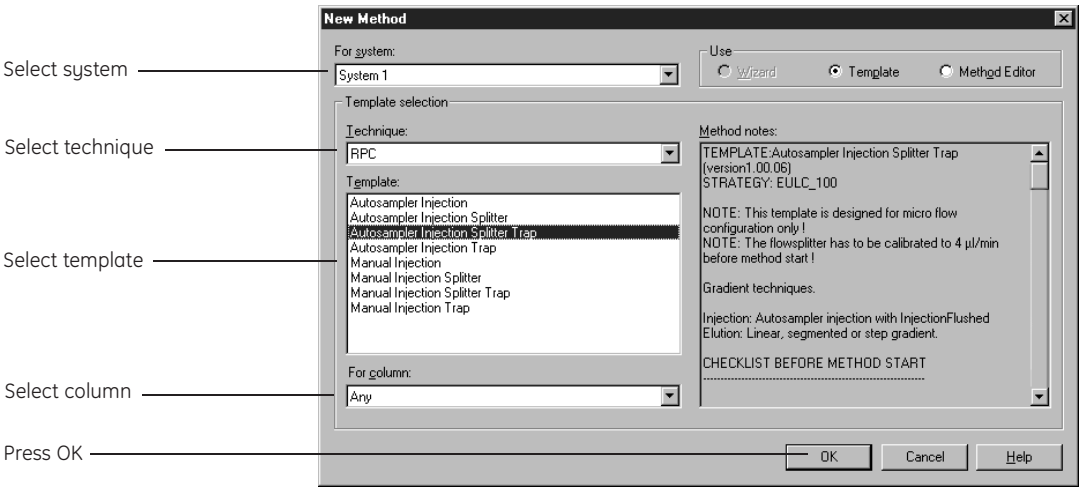
Comprehensive on-line help is available. To get help about an instruction or module, place the cursor on the instruction/module and press the **F1** key. Alternatively, click on the **Help** menu in the upper right corner of each module and select **Help for...** to get general help about the current instruction or module and find new help topics, or **Help Index** for a specific topic. Double-click on green text to get more help. In any box, click on the **Help** menu to get help on how to use the current active box.

3 Creating a method

UNICORN is supplied with a number of almost ready-made methods called method templates. The method templates can be run as they are or you can easily modify them to design your own method in a very short time.

The following example shows how to create a method when using autosampler injection, the flow splitter and a trap column:

1 Click on in the Main menu Toolbar. The **New Method** window will appear.



2 Select a system. Then select **RPC** as chromatographic technique.

3 A list of available templates will appear. By clicking on a template, an explanation for the template appears to the right in the **Method Notes** field. Select the template **Autosampler Injection Splitter Trap.**

Comment:

The other templates are briefly described at the end of this chapter.

4 Select the column you intend to use. The correct column volume, the recommended flow rate, and the correct pressure limit for that column will then be automatically implemented in the method.

Comment:

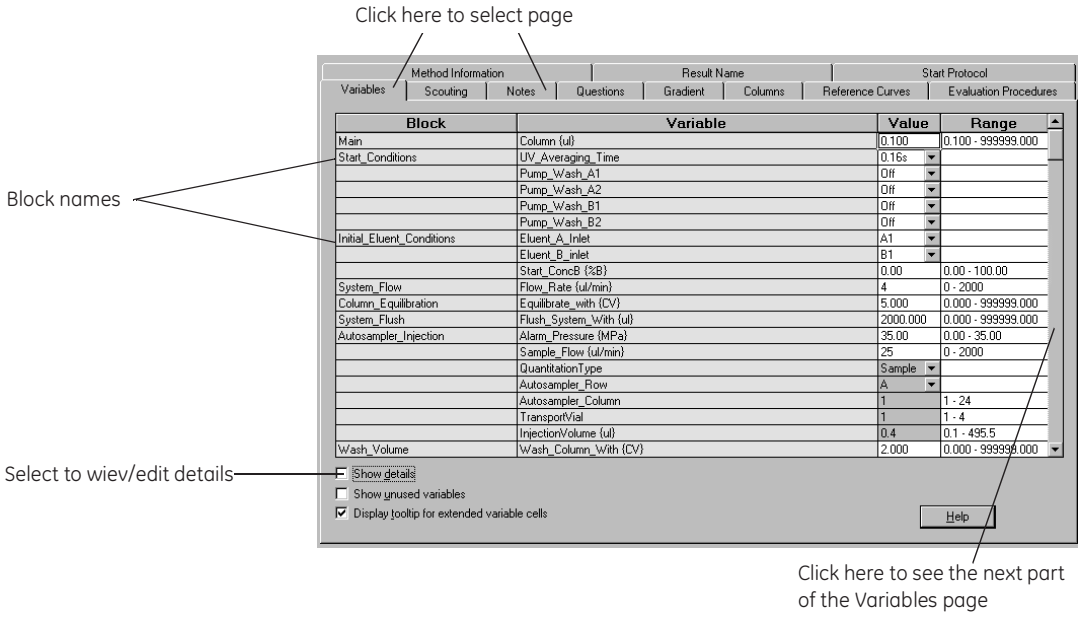
If you do not find your column in the list, you can add one. See the UNICORN User Manual.

If you manually alter the default values, and thereby exceed the recommended values for the selected column, you will get a warning when saving your method.

If you want to perform a test run without a column, select **Any** from the column list to get suitable default parameters in the method. Then, in the method, use a piece of tubing to replace the column.

5 Click **OK.** The method template will now be opened as an untitled method.

6 Choose **View:Run Setup** (may already be checked).



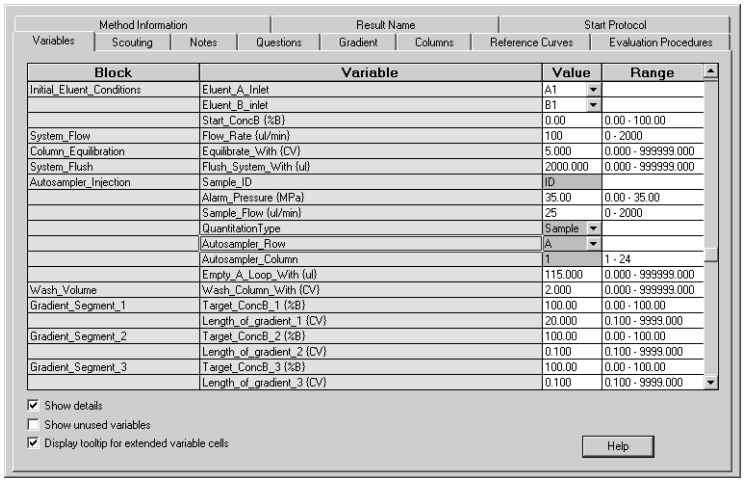
7 Run Setup consists of a number of pages. You will only look at **Variables** and **Notes** now. You select a page by clicking on the respective tab at the top of the Run Setup screen.

8 On the **Variables** page, the method is presented by a number of Blocks. The Blocks represent the typical steps in a chromatographic run:

- Start conditions
- Column equilibration
- Autosampler injection
- Wash out unbound sample

- Gradient
- Clean after gradient
- Re-equilibration

Each block contains a number of Method variables with suitable default values. The values are easily changed to suit your requirements. Some of the variables are normally hidden but can be shown by checking the **Show details** box. Click on the scroll bar to see the next part of the **Variables** page.



9 To ensure complete sample application onto the trap column, the sample volume value must be appropriate for the autosampler loop used. Check that the variable **Empty_loop_with** in the block **Autosampler_Injection** is set to five times the volume of the sample loop.

In the template **Autosampler Injection Splitter Trap,** the default value is set to 115 µl, which is an appropriate value for the default autosampler loop (20 µl x 5 + 15 µl).

10 The length of a gradient segment is limited to 9999 volume or time units (µl or min). To run longer gradients than 9999 units, use additional gradient segments so that each segment does not exceed 9999 units. Define the additional segments on the **Variables** page. Enter desired values in the variables **Length_of_Gradient** respective **Target_ConcB** in the block **Gradient_Segment.**

Note: If using the flow splitter (SplitterFlow as Method Base), the gradient segment limit is decreased according to how much the flow splitter reduces the system flow. For example, if the system flow is 100 µl/min and the split flow is 4 µl/min, the gradient segment limit will be 399 µl or min. (9999/25).

Comment:

Each method template is unique, but they are all built up with the same principle. Below is a description of all the blocks in the **Autosampler Injection Splitter Trap** template.

- **Main**
  The column selected is shown here.

- **Start Conditions**
  Defines the general settings during a run. Wavelengths not used are set to zero. In the column list, typical peak width and max pressure are pre-defined. UV averaging time and column pressure limit are automatically set to a default value for the column selected.

- **Initial Eluent Conditions**
  Select the eluent inlets (default A1 and B1, respectively). Set the start concentration of eluent B (default 0%B). The flow rate is set equal to the default value of the selected column.

- **System Flow**
  The default "Flow rate" value is set to 100 µl/min. To get the desired flow rate for the selected column, calibrate the flow rate from **System:Settings:Special.**

- **Column Equilibration**
  The number of column volumes (CV) required to equilibrate the column is set here. An autozero of the UV monitor is done at the end of equilibration.

- **System Flush**
  Washes the mixer with set B-buffer concentration. Default value is **2000 µl for the 0.2 ml mixer.**

- **System Volume Compensation**
  Make sure that the correct mixture of A and B-buffer reaches the column, by compensating for the dead volume in the system.

- **Pressure delay**
  Allows the pressure to decrease before injecting the sample.

- **Autosampler Injection**
  Injects the sample from a selected vial in the autosampler. To apply all the sample onto the trap column, empty the sample loop with a volume 5 times the volume of the sample loop + additional 15µl. (If using the default autosampler loop (20 µl).
  The method uses scouting to facilitate automatic injection of several samples or standards. Set the following variables on the **Scouting** page: **Sample_ID, Quantitation_Type, Autosampler_Row** and **Autosampler_Column.** Replace the default text (ID) in **Sample_ID** to allow individual identification of all runs. Select the vial that contains the sample. For automatic quantification,
  set **Quantitation_Type** to **Sample** or the appropriate standard level. Ignore **QuantitationType** unless the run is intended for quantification.

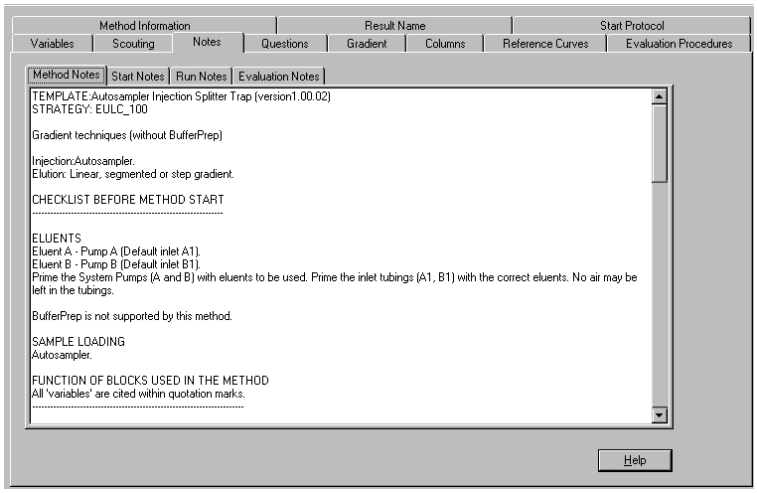
- **Wash out Unbound Sample**
  To wash out unbound sample with the start eluent, set the length of the wash here.

Gradient Elution

- **Gradient Segment 1, 2 and 3**
  The start concentration of eluent B, the end concentration and the length of the gradient is set here. A linear gradient is created. Three separate gradients can be defined. A step gradient is defined by setting the concentration of eluent B and zero length in one segment.

- **Gradient delay**
- **Clean after Gradient**
  Set the concentration of eluent B for column cleaning and the duration (in column volume units) here.
- **Flow between runs**
  Allows the flow to continue between the runs.

11 Click on the **Notes** tab.



The method notes contain comments and information about the method, e. g. how sample injection and elution are performed.

12 Select **File: Save.** The **Save As** dialog opens. Enter a name. Store the method in the directory of your choice by double-clicking on a directory. Click **OK.**

Comment:

The method name, followed by two consecutive numbers starting with 01 will then be used as default name for the result file of your method after runs.

13 You have now created a method.

14 Click on the UNICORN menu icon at the bottom of the screen. Your saved method appears in the window to the left.

Now you are ready to start a run. Go to chapters 4 and 5.

You can also go to chapter 8 to learn how to vary any variables systematically and automatically in repeated runs. This is know as scouting and is a convenient, easy to use function.

3.1 Template naming conventions

All method templates (except the Installation test) are named according to its application. The template name consists of three parts where each part describes the use of a specific type of application.

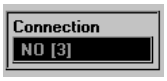
Example: **Autosampler Injection Splitter Trap.**

Type of	Description	Example (description)
Injection	Specifies used sample application method	**Manual Injection** (the sample loop is filled manually with a syringe) **Autosampler Injection** (automatic sample application with an autosampler)
Splitter	Specifies use of a flow splitter	**Splitter**
Trap	Specifies use of a trap column	**Trap**

4 Preparing the system for a run

4.1 System connection

1 Click on the **1. System Control** button in the Task bar at the bottom of the monitor.

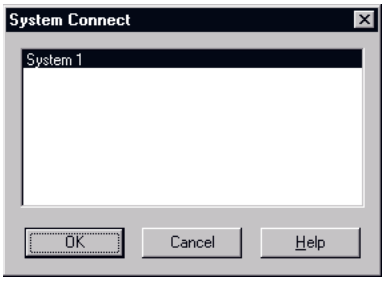


2 If the text **NO** is written in the **Connection** panel in the **Run Data** window, continue below. If it says **YES**, go directly to *General system preparation*.

Comment:

Before you can start a run, you must always connect to the system. Connecting means that the System Control window is set up for a particular system. If you are not connected, the text **NO** is written in the **Connect** panel in the **Run Data** window. Once you are connected, the text changes to **YES**.

3 Select **System:Connect....** The **System connect** dialog appears:



4 Select a system from the list. If you are not connected to a network, only one system will be shown. Click **OK**.

5 When connected, the text **YES** is written in the **Connect** panel in the **Run Data** window. You only have to connect once. If you do not select **System:Disconnect**, you will be automatically connected to the system the next time you login to UNICORN.

4.2 General system preparation

4.2.1 Checking the tubing

1 The correct tubing kit for the column you intend to use must be installed (i.d. 75 µm or 150 µm). See *Chapter 2* in the *Ettan microLC System Manual* for an overview over columns with recommended tubing kits.

Comment:

If tubing with too large inner diameter is used, the peaks will become broader than necessary. If tubing with too small inner diameter is used, the backpressure from the tubing might become higher than the maximum pressure for the column and the run will stop immediately after it is started.

2 Immerse inlet tubing A1 (or A2 if you changed this in the method) in buffer A and inlet tubing B1 in buffer B.

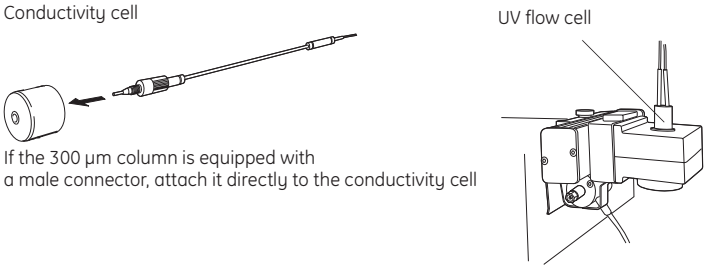
3 Check that the outlet tubing from the UV cell is put into a waste bottle, or connected to an MS unit (if used).

4 If there is air in the inlet tubing, or if you suspect air in the pump, purge the pump with a syringe as described in *Pump P-905 User Manual*.

4.2.2 Connecting the columns

1 Connect the trap column (if used) between the flow direction valve, port 3 and the injection valve, port 1. Use the S7 capillary and 1/16"/1/16" male union or the tubings delivered with the trap column.

2 Connect the separation column between the conductivity cell and the top of the UV flow cell.


Conductivity cell
UV flow cell
If the 300 µm column is equipped with
a male connector, attach it directly to the conductivity cell

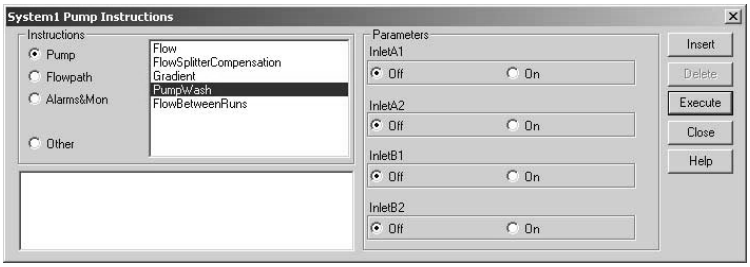
Follow tubing recommendations as follows.

Column	Tubing (i.d)	Length
300 µm	Silica 75 µm	450 mm
≥1.0 mm	PEEK 150 µm	300 mm

4.2.3 Preparing the pump

1 Click **System Control** to open the **System Control** window.

2 Select **Manual:Pump**. **The Pump Instructions** dialog opens.



3 Select instruction **PumpWash.** Select **ON** for the inlet tubing that should be filled. Select **OFF** for the non-used inlet tubing.

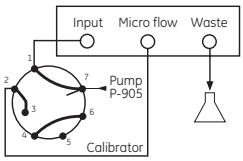
4 Click **Execute** to fill the inlet tubing with correct solutions. The flow valve will automatically switch to waste during the **PumpWash.**

5 When the **PumpWash** is finished, click **Close.**

6 Click **End** in the **System Control** window.

7 Make sure that the correct compression compensation values are set in Pump P-905. This is described in *Pump P-905 User Manual*.

4.2.4 Calibrating the flow splitter (if used):



1 Connect the port 1 on the flow direction valve to the **Input** port of the splitter. Use the tubing marked **P3.**

2 Connect the enclosed waste tubing to the **Waste** port.

3 Connect the calibrator **CAL-100-0.3,** included with the system, between the **Micro flow** port on the flow splitter and port 2 on the flow direction valve.

4 Connect a measurement syringe to the UV cell outlet tubing.

5 If not already open, open the **System Control** window.

6 Measure the flow rate after the column:

$$\text{Flowrate}\left[\frac{\mu l}{\text{min}}\right] = \frac{\text{volume}[\mu l]}{\text{time}[\text{min}]}$$

7 Choose **System:Settings...** The **Settings** dialog opens.

8 Select **Special** in the **Instructions** field, and then **FlowsplitterParameter.**

9 Enter the desired and the measured flow rates.

10 Click **OK.** The calculation takes place.

11 When running the pump manually, the flow splitter compensation must be switched on in **Manual:Pump.** Select **On** in **FlowSplitterCompensation.**

12 Measure the flow rate again.

4.2.5 Initializing the microplate

The plate type used must be entered in UNICORN.

1 Open the **System Control** window if it is not already open.

2 Choose **System:Settings...** The Settings dialog opens.

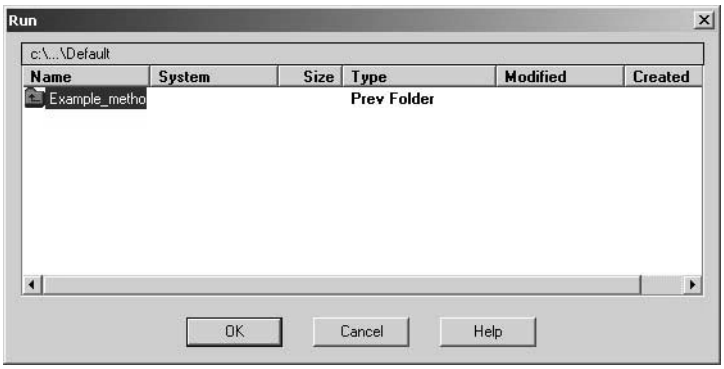
3 Select Special in the **Instructions** field, and then **Autosampler:Parameters:PlateType.**

4 Select the plate type used.

5 Starting a run

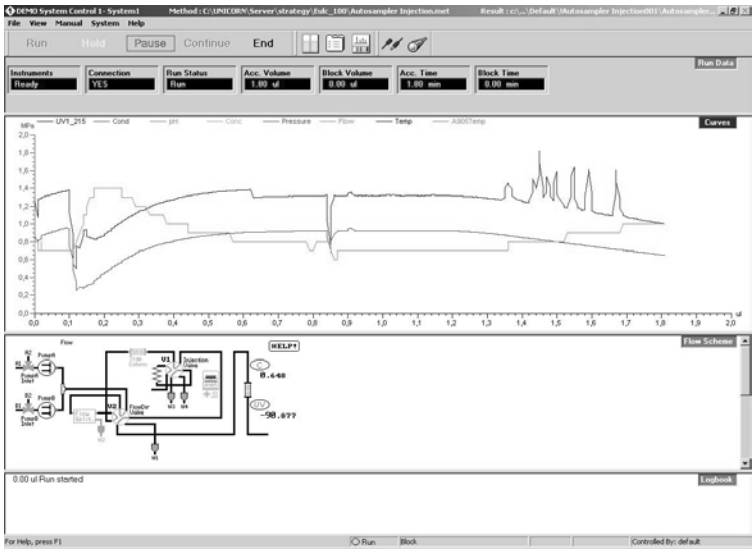
1 Open the **System Control** window, if it is not already open.

2 Choose **File:Run...** Select the method to start. Click **OK.** The run starts.



6 Viewing a run

The progress of the run is shown in the **System Control** window.

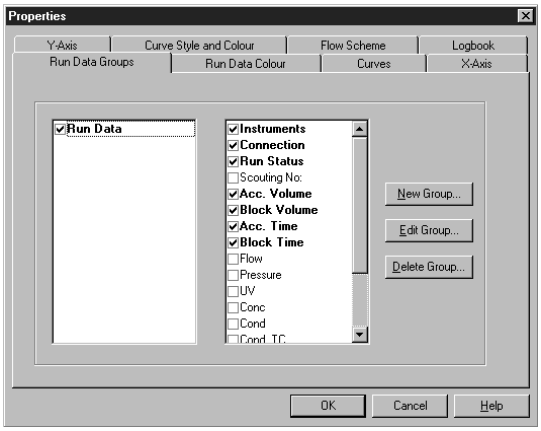


When the system pump is running, the text **Run** is shown in the **Run Status** panel in the **Run Data** window.

1 Choose **View: Windows.** Check **Rundata, Curves** and **Logbook**. Click **OK.**

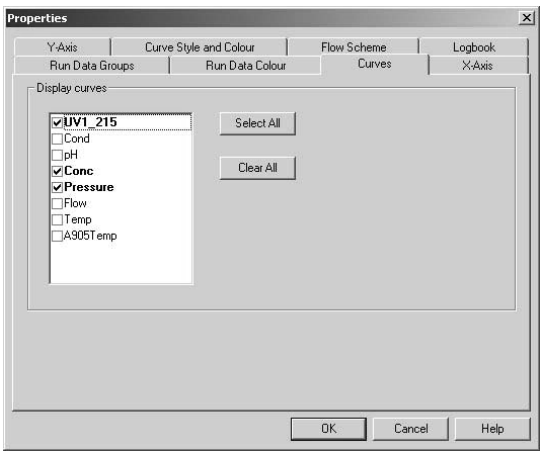
2 The **Run Data** window at the top shows current values for running parameters.

3 Position the cursor in the **Run Data** window. Click the right mouse button and select **Properties...** The **Properties** dialog opens. Now you can select which parameters you want to see in the **Run Data** window. For example, select **Acc. time, Block time, Flow** and **Pressure.** Click **OK.**



4 The **Curves** window shows the curves during the run.

5 Position the cursor in the **Curves** window. Click on the right mouse button and select **Properties....** The **Properties** dialog opens. Here you can select which curves to show during the run. All curves are stored in the result file.



6 By clicking the different tabs in the **Curve Properties** window you can set the properties for the different curves. Normally the curves are scaled with auto scaling, i.e. the scale is adjusted continually to the highest and lowest values for each curve.

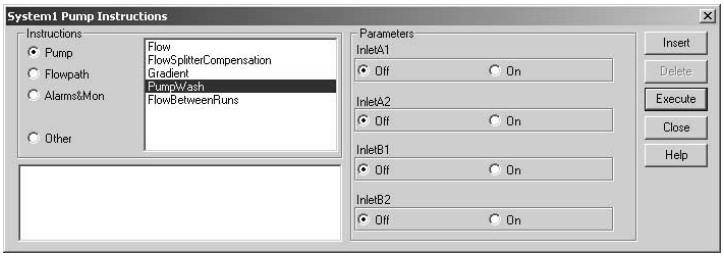
7 To fix the Y-axis scale for a curve, mark the curve, click on **Y-axis,** click on **Fixed,** and enter the max. and min. values. You can repeat this for other curves. Click **OK.**

8 To maximize the **Curve Data** window, position the cursor in the **Curve Data** window. Click the right mouse button and select **Maximize.** Go back to normal size by clicking on **Restore.**

9 Click on the Y-axis scale, or click on the curve name at the top of the **Curve Data** window to shift to a scale for another curve. The colour of a curve, its Y-scale, and its name are always the same. Click on the **X-axis** to shift between time and volume.

10 The **Logbook** is shown at the bottom. The **Logbook** shows exactly when the instructions in the method are executed during the run. The **Logbook** is stored in the result file.

11 You can make manual changes during the run. Select **Manual:Pump.** The **Pump Instructions** dialog opens.



12 If, for example, you want to change the flow rate, select **Pump** and then **Flow.** Enter a new flow rate under **Parameters** and then click **Execute.** The new flow rate will be used until the end of the run or until a new flow rate instruction is reached in the method. Close the box by clicking **Close.** All manual interactions are recorded in the **Logbook.**

13 If you want to stop the run before it is finished, click on the **End** button at the top.

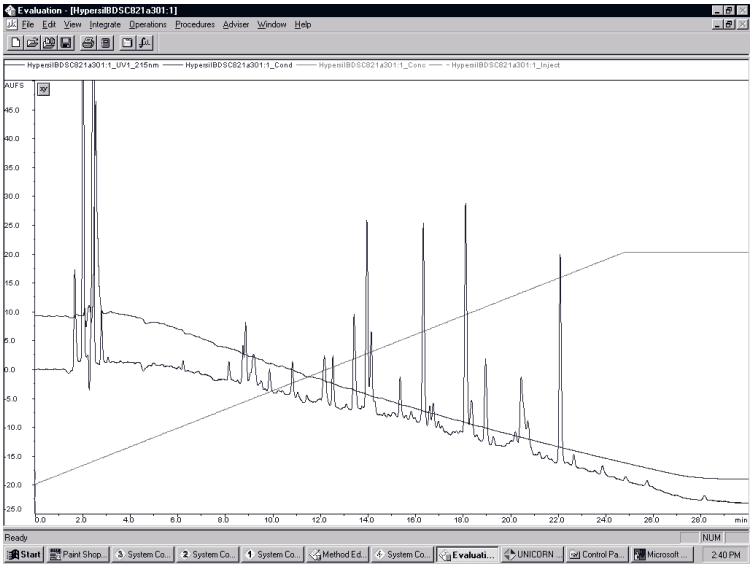
7 Viewing and printing the result

If you are satisfied with the automated print-out obtained after the run (if selected), you need not alter anything described in this section. However, if you want to alter the chromatogram layout, this section will teach you the basics of the evaluation module.

7.1 Viewing

1 After a run you can view the result. Click the UNICORN menu icon. Double-click on a result file icon in the list to the right.

2 The **Chromatogram** window is opened automatically in the Evaluation workspace when you open a result file. The **Chromatogram** window contains all the curves. Note that the term chromatogram is used here when talking about the whole window containing all the different curves.



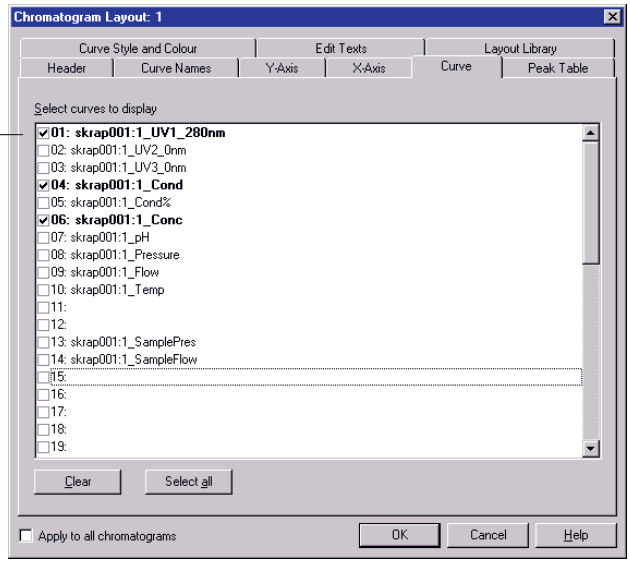
The result file from a run holds a complete record of the run, including method, system settings, curve data and run log.

Comment:

Original raw data curves can never be modified, renamed, or deleted from a result file.

3 Maximize the **Chromatogram** window by clicking on the larger square in the upper-right corner.

4 All changes regarding the presentation of the curves are done in the **Chromatogram Layout** window. Position the cursor in the **Chromatogram** window. Click on the right mouse button and select **Properties....,** or select **Edit: Chromatogram layout...** to activate this window.



5 Highlight the curves to view under **Curves.** Curves are named as **Resultfile:1_"curve"** where a curve can be, for example, UV_wavelength, Cond, pressure...etc. De-select all curves except, for example, the UV, Cond and Conc. curves. Click **OK** at the bottom of the **Chromatogram Layout** window.

6 You can easily zoom in on the curves. Place the cursor in the chromatogram, press the left mouse button and drag a rectangle. When you release the mouse button, the part within the rectangle will be enlarged. You can zoom further on the enlarged part. Click the right mouse button and choose **Undo** or **Reset zoom** to return to the complete chromatogram.

7 Click on the **Y-axis** scale to change to a scale for another curve. The style and colour of a curve, its Y-scale and its X-scale can all be changed.

8 Open the **Chromatogram Layout** window again. Click on the **Y-axis** and **X-axis** tabs to set the scale for the different curves. Normally, the curves are scaled with auto scaling, i.e. the highest and lowest values for each curve set the scale.

9 To fix the Y-axis scale, mark a curve, click **Fixed,** and enter the max. and min. values for that curve. You can repeat this for other curves.

10 To fix the X-axis scale, click **Fixed** in the X-axis field, and enter the min. and max. values for the X-axis. Click **OK.**

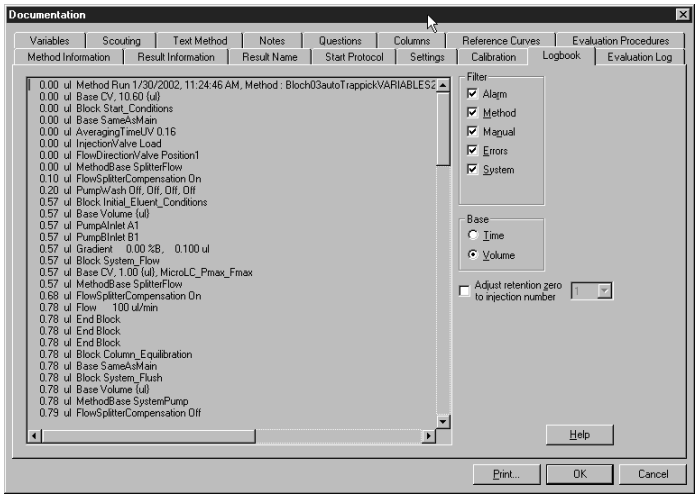
11 Click **OK** at the bottom of the **Chromatogram Layout** window to execute all the changes.

Comment:

When you have made the necessary changes in the Chromatogram layout box, they can be saved as a layout. Click **Save as** at the top of the **Chromatogram layout** dialog to save the layout. Give the layout a name and click **OK.** Layouts can be selected in **Apply layout** at the top of the box and all your saved selections will apply. Saved layouts can be applied to any result file.

12 Minimize the chromatogram window by clicking on the smaller squares in the upper-right corner.

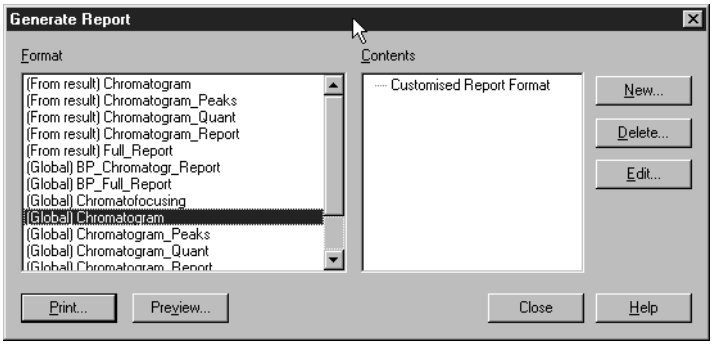
13 Click **View Documentation.** A number of pages appear as in the Run Setup in the Method Editor. All documentation about the run is stored here, e.g. the method, answers to questions, variables, logbook...etc. For example, click on the **Notes** and **Logbook** pages to check the contents. Close the **Documentation** window by clicking on the **X** in the upper right corner.



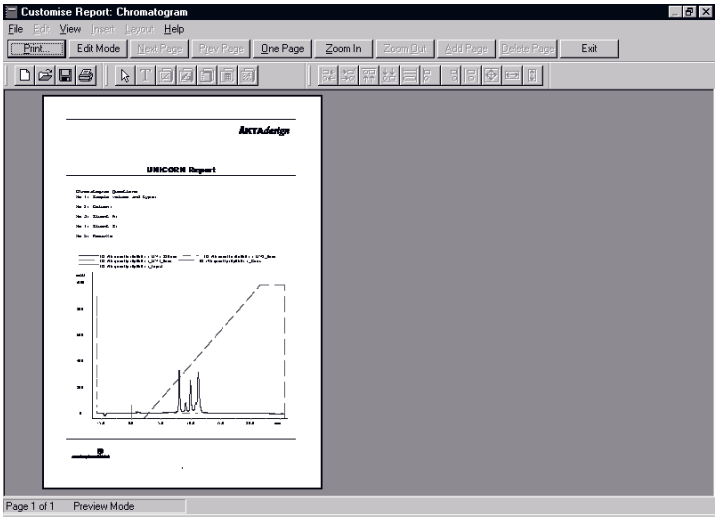
7.2 Printing and making a report

1 To print the chromatogram, choose **File:Report.**

2 Select standard format **(Global) Chromatogram.** This will create a report containing the chromatogram and the questions on one page.



3 Click **Preview** to view the report on the screen.



4 Click **Edit Mode** to enable changes in the report.

5 To include more information in the report, click **Add Page.** A new page is added to the report.

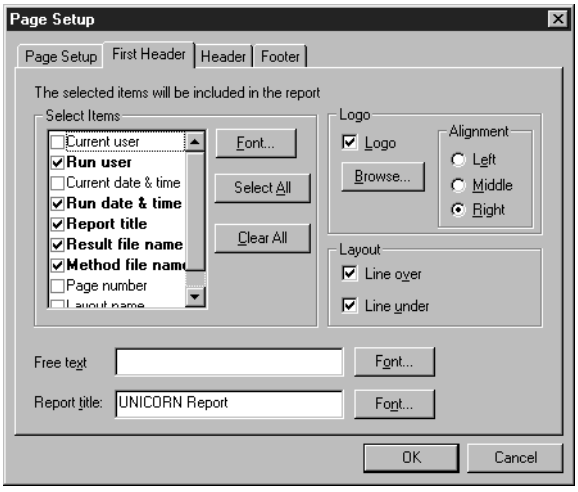
6 Under **Insert,** click on the item to include. Items available are:

- Free text
- Chromathogram
- Text method
- Documentation
- Evaluation log
- Quantitate and molsize (optional)

7 Move the mouse pointer into the page area of the window. You will notice that the mouse pointer has an additional symbol according to the item type you selected to insert.

8 Press the left mouse button and drag out a box to the desired size. Release the mouse button. A dialog is displayed specific to the type of item inserted. Make the appropriate selections in the dialog and click **OK** to view the inserted item.

9 If you want to change the page layout, choose **Edit: Page Setup.** The **Page Setup** window is opened and you can, for example, select page size and items to be included in the header and in the footer. The information selected here will be printed in the report. Click **OK** at the bottom of the **Page Setup** window.



10 Click **Print** to print the report.

8 Scouting

Scouting allows any run parameters, e.g. flow rate, to be systematically varied automatically, in repeated runs.

Below is a description of how to perform scouting.

1 Open the Main menu window. Choose **File:New:Method.** The **New Method** dialog opens. Select system. Then select **RPC** as technique.

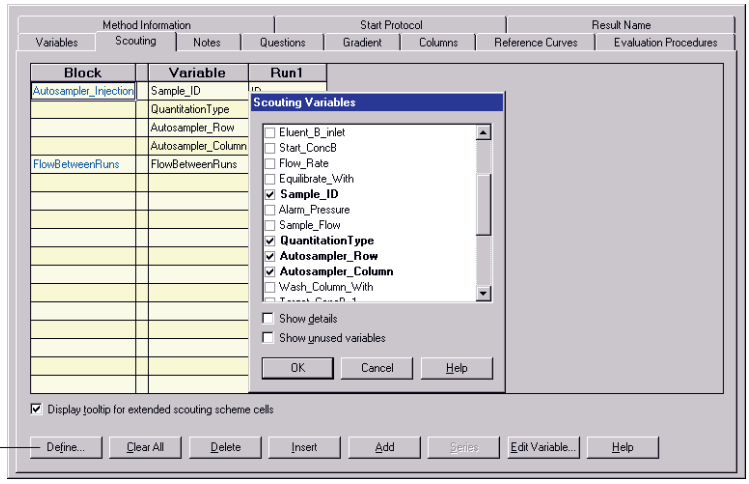
2 Select the desired template, for example, **Autosampler Injection Splitter Trap.** Select a column and click **OK.**

Comment:

A special template is not required for scouting. Scouting can be performed with any template for any technique.

3 Choose **View:Run Setup** in the **Method editor** (may already be checked).

4 Select the **Scouting** page.



Defining other scouting variable

5 A list of all the variables appears. Select the block **Autosampler_Injection** and the variable you wish to alter.

6 Click **OK.** The selected scouting variables will appear to the left with their default values inserted.

Comment:

Values for variables selected for scouting are dimmed on the Variables page and cannot be changed there.

7 To change a variable value, position the cursor in the value field and double-click with the left mouse button.

8 To add a column for next run, click **Add.** A second column appears with the values from the previous run copied. Change the values as required.

If you want to insert a new run column after a specific column in the scouting scheme, position the cursor in the column and click **Insert.** A new column with identical values appears directly after the selected column.

9 Repeat the previous step until you have defined all the runs you require. If necessary, use the horizontal scroll bar to see more runs.

10 Click on **Run1, Run2,** etc. at the top of the scheme with the right mouse button to toggle between **Run** and **Excluded** for the different runs. Those marked **Excluded** will not be run. A scouting scheme is now defined.

11 Select the **Start Protocol** page. To stop the system between each run for e.g. refilling the sample loop, check the **All runs** box. Otherwise, the system will not stop until all runs are finished.

12 Choose **File:Save.**

13 When the method is started all the runs in the scheme will be performed automatically and the set flow for each run will be prepared automatically. Each run in the scouting scheme will generate a separate result file which are all stored in a special scouting directory.

14 Prepare the system and start the run as described in chapters 4 and 5.

15 When filling the inlet tubing with the correct solutions, use the instruction **PumpWash** in **System Control:Manual:Pump.** Select **ON** for the inlet tubings that should be filled, and select **OFF** for the nonused tubings. Click **Execute** to fill the inlet tubing. When the **PumpWash** is finished, click **Close.**

9 Going further

Once you are used to the system and software you may want to learn more about it and its capabilities. Below is a list of operations and descriptions that you may find of interest, they are cross-referenced to other manuals in the Ettan microLC manual package.

To learn about	Read
Columns and recommended tubing	Ettan microLC System Manual
Changing tubing kits	Ettan microLC System Manual
Calibrating monitors and pumps	UNICORN 5.0 User Manuals
Comparing chromatograms	UNICORN 5.0 User Manuals
Intergrating curves	UNICORN 5.0 User Manuals
Measuring HETP and resolution	UNICORN 5.0 User Manuals
Exporting curves and data to other programs	UNICORN 5.0 User Manuals
Finding information about a certain menu instruction in UNICORN	Click on Help button in the dialog box that appears or look in the index in the UNICORN 5.0 User Manuals
Controlling Pump P-905 and Monitor UPC-900 from the dials on the instruments themselves	Ettan microLC System Manual to unlock the dials. The User Manual for each instrument, found in the binderEttan microLC Components
Details about each component	See each individual manual in the binder Ettan microLC Components
Security features	UNICORN 5.0 User Manuals
Controlling the system from a remote computer	UNICORN 5.0 User Manuals

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Short instructions

The following short instructions are intended as a guide for users who are fully familiar with the safety precautions and operating instructions described in this manual. The instructions assume that the unit is installed according to the installation instructions.

1 Choose **File:New:Method** in the main menu or click on

2 Select **System, Technique, Template** and **Column**. Click **OK.**

3 Choose **File:Save** in the **Method** editor and give the method a name. Click **OK**.

4 Click on

5 Choose **File:Run.** Select the method and click **Run**.

6 The start protocol will appear. Check the method on the **Variables** page and change values as you require.

7 Click the **Next** button and go through all the other pages.

8 On the **Evaluations procedures** page, select Print_Chromatogram to get a print-out automatically after the run.

9 Click the **Start** button on the last page and the run will start.

For contact information for your local office, please visit
www.gelifesciences.com/contact

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

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GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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